

WHAT IS CLAIMED IS:

1. A method of evaluating a compound for the ability to inhibit binding of an intracellular receptor region of an α -subunit of a voltage-gated ion channel and an amino-terminal inactivation region of an ion channel protein, comprising:

(a) contacting the compound with said intracellular receptor region and said amino-terminal inactivation region; and

(b) determining the ability of said compound to interfere with the binding of said intracellular receptor region with said amino-terminal inactivation region, wherein a decrease in said binding in the presence of said compound compared to said binding in the absence of said compound indicates that said compound inhibits binding of said intracellular receptor region to said amino-terminal inactivation region.

2. The method of claim 1, wherein the voltage-gated ion channel is a potassium channel or a sodium channel.

3. The method of claim 1, wherein the intracellular receptor region comprises an S4-S5 cytoplasmic receptor domain of an α -subunit of a voltage-gated channel protein, or a biologically active fragment thereof.

4. The method of claim 3, wherein the voltage-gated channel protein is a potassium channel protein selected from the group consisting of Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, Kv1.6, and Kv3.4.

5. The method of claim 1, wherein the amino-terminal inactivation region comprises an amino-terminal domain of a potassium channel protein or a sodium channel protein, or a biologically active fragment thereof.

6. The method of claim 5, wherein the potassium channel protein is selected from the group consisting of Kv β 1, Kv β 1.2, Kv β 1.3, Kv β 3, Kv1.4, and Kv3.4.

7. A method of screening a candidate compound for the ability to inhibit binding of an intracellular receptor region of an α -subunit of a voltage-gated ion channel to an amino-terminal inactivation region of an ion channel protein, comprising:

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(a) adding said candidate compound to a modified host cell comprising a reporter gene; and

10 (b) monitoring expression of said reporter gene, wherein a decrease in expression is an indication that said candidate compound inhibits binding of the intracellular receptor region of the α -subunit to the amino-terminal inactivation region of the ion channel protein.

15 8. The method of claim 7, wherein the voltage-gated ion channel is a potassium channel or a sodium channel.

20 9. The method of claim 7, wherein the voltage-gated ion channel is a potassium channel protein selected from the group consisting of Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, Kv1.6, and Kv3.4.

10. The method of claim 7, wherein the amino-terminal inactivation region is an amino-terminal domain of a potassium channel protein selected from the group consisting of Kv β 1, Kv β 1.2, Kv β 1.3, Kv β 3, Kv1.4, and Kv3.4.

25 11. A modified host cell comprising:

30 (a) a first hybrid protein comprising a DNA-binding domain of a transcriptional activator in polypeptide linkage to either (i) an intracellular receptor region of an α -subunit of a voltage-gated ion channel or (ii) an amino-terminal inactivation region of an ion channel protein; and

(b) a second hybrid protein comprising an activation domain of a transcriptional activator in polypeptide linkage to said intracellular receptor region if said DNA-binding domain is in polypeptide linkage to said amino-terminal inactivation

region or to said amino-terminal inactivation region if said DNA-binding domain is in polypeptide linkage to said intracellular receptor region.

12. The modified host cell of claim 11, wherein the voltage-gated ion
5 channel is a potassium channel or a sodium channel.

13. The modified host cell of claim 11, wherein the intracellular receptor
region is an S4-S5 cytoplasmic receptor domain of a potassium channel protein
selected from the group consisting of Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, Kv1.6, and
10 Kv3.4.

14. The modified host cell of claim 11, wherein the intracellular receptor
region of an α -subunit comprises an amino acid sequence selected from the group
consisting of:
15

(a) an amino acid sequence as set forth in SEQ ID NO:1, or a biologically
active fragment thereof;

(b) an amino acid sequence as set forth in SEQ ID NO:2, or a biologically
20 active fragment thereof; and

(c) an amino acid sequence which is at least 90 to 95% identical to the
amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2.

15. The modified host cell of claim 11, wherein the amino-terminal
inactivation region is an amino-terminal domain of a potassium channel protein or a
sodium channel protein.

16. The modified host cell of claim 11, wherein the amino-terminal
30 inactivation region is an amino-terminal domain of a potassium channel protein
selected from the group consisting of Kv β 1, Kv β 1.2, Kv β 1.3, Kv β 3, Kv1.4, and Kv3.4.

17. The modified host cell of claim 11, wherein the amino-terminal inactivation region comprises an amino acid sequence selected from the group consisting of:

5 (a) an amino acid sequence as set forth in SEQ ID NO:5, or a biologically active fragment thereof;

(b) an amino acid sequence as set forth in SEQ ID NO:6, or a biologically active fragment thereof; and

10 (c) an amino acid sequence which is at least 90 to 95% identical to the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6.

18. The modified host cell of claim 11, wherein said host cell is selected from the group consisting of a yeast cell, a mammalian cell, an amphibian cell, and a bacterial cell.

19. The modified host cell of claim 18, wherein said yeast cell is selected from the group consisting of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Pichia pastoris*.

20. The modified host cell of claim 11, wherein the transcriptional activator is selected from the group consisting of Gal4, Gcn4, Hap1, Adr1, Swi5, Ste12, Mcm1, Yap1, Ace1, Ppr1, Arg81, Lac9, Qa1F, VP16, LexA, and a mammalian nuclear receptor.

21. The modified host cell of claim 11, wherein the transcriptional activator is Gal4.

22. The modified host cell of claim 11, further comprising a reporter gene whose transcription is dependent upon the first hybrid protein and the second hybrid protein being bound to each other, thereby reconstituting a transcriptional activator.

23. The modified host cell of claim 22, wherein the reporter gene is selected from the group consisting of:

- 5 (a) genes conferring sensitivity to a chemical;
 (b) genes conferring resistance to a chemical;
 (c) genes complementing auxotrophies; and
 (d) *LACZ*, Luciferase gene, green fluorescent protein gene, *URA*, *CAT*, *LAC1*, and *GAL80*.

10 24. The modified host cell of claim 22, wherein the reporter gene is a *HIS* gene or a *CYH2* gene.

25. The modified host cell of claim 22, wherein
 15 the first hybrid protein comprises a Gal4 DNA-binding domain in polypeptide linkage to an S4-S5 cytoplasmic receptor domain of an α -subunit of a Kv1.1 channel protein, or a biologically active fragment thereof;

the second hybrid protein comprises a Gal4 activation domain in polypeptide
 20 linkage to the amino-terminal inactivation region of a Kv β 1 cytoplasmic protein, or a biologically active fragment hereof; and

the reporter gene comprises *CYH2*.

25 26. The modified host cell of claim 22, wherein

the first hybrid protein comprises a Gal4 DNA-binding domain in polypeptide linkage to an S4-S5 cytoplasmic receptor domain of an α -subunit of a Kv1.4 channel protein, or a biologically active fragment thereof;

30 the second hybrid protein comprises a Gal4 activation domain in polypeptide linkage to the amino-terminal inactivation region of an α -subunit of an Kv1.4 channel protein, or a biologically active fragment hereof; and

the reporter gene comprises CYH2.

27. The modified host cell of claim 22, wherein the modified host cell is a yeast cell derived from a *Saccharomyces* organism having the genotype MATa,
 5 *gal80, gal 4, his3, ade2-101, leu2-3, 112 trp1-901, ura3-52 cyh^r LYS2::GAL_{UAS}-HIS3.*

28. A method for identifying compounds which inhibit N-type inactivation of a voltage-gated ion channel, comprising:

(a) administering a compound to the modified host cell of claim 22 and
 10 incubating the modified host cell for a suitable period;

(b) determining whether the administration of the compound inhibits expression of the reporter gene; and

15 (c) identifying a compound which inhibits expression of the reporter gene as an inhibitor of N-type inactivation of said voltage-gated ion channel.

29. A modified host cell comprising:

20 a first hybrid protein comprising an intracellular receptor region of an α -subunit of a voltage-gated ion channel in polypeptide linkage to a first peptide of a peptide binding pair; and

25 a second hybrid protein comprising an amino-terminal inactivation region of an ion channel protein in polypeptide linkage to a second peptide of the peptide binding pair;

30 wherein binding interaction between the first peptide and the second peptide in the modified host cell causes activation of a signal transduction pathway in said modified host cell.

30. The modified host cell of claim 29, wherein the voltage-gated ion channel is a potassium channel or a sodium channel.

31. The modified host cell of claim 29, wherein the intracellular receptor region is an S4-S5 cytoplasmic receptor domain of a potassium channel protein selected from the group consisting of Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, and Kv3.4.

5 32. The modified host cell of claim 29, wherein the amino-terminal inactivation region is an amino-terminal domain of a potassium channel protein selected from the group consisting of Kv β 1, Kv β 1.2, Kv β 1.3, Kv β 3, Kv1.4, and Kv3.4.

10 33. The modified host cell of claim 29, wherein said host cell is selected from the group consisting of a yeast cell, a mammalian cell, an amphibian cell, and a bacterial cell.

15 34. The modified host cell of claim 33, wherein said yeast cell is selected from the group consisting of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Pichia pastoris*.

20 35. The modified host cell of claim 29, wherein said first peptide of the peptide binding pair is either an effector molecule or a cell compartment localization domain, and wherein said second peptide of the peptide binding pair is (i) a cell compartment localization domain if said first peptide is an effector molecule or (ii) an effector molecule if said first peptide is a cell compartment localization domain.

25 36. The modified host cell of claim 35, wherein said effector molecule is a guanine nucleotide exchange factor and said cell compartment localization domain is a plasma membrane localization domain.

30 37. The modified host cell of claim 36, wherein said guanine nucleotide exchange factor is an SOS and said plasma membrane localization domain is a myristoylation signal.

38. The modified host cell of claim 35, wherein said effector molecule activates an indicator molecule selected from the group consisting of a MAP kinase, a RAS protein, a JAK protein, a JNK protein, and IRS-1 protein.

39. A polynucleotide encoding a DNA-binding domain or an activation domain of a transcriptional activator and comprising a nucleotide sequence selected from the group consisting of:

- 5 (a) the nucleotide sequence of SEQ ID NO:3;
- (b) the nucleotide sequence of SEQ ID NO:4;
- (c) a nucleotide sequence which is at least 90% identical to the nucleic acid of (a) or (b) and which encodes a peptide that is capable of binding to an amino-terminal inactivation region of an ion channel protein; and
- 10 (d) a nucleotide sequence which is degenerate as a result of the genetic code to a nucleic acid defined in (a) or (b) and which encodes a peptide that is
- 15 capable of binding to an amino-terminal inactivation region of an ion channel protein.

40. A polynucleotide encoding a DNA-binding domain or an activation domain of a transcriptional activator and comprising a nucleotide sequence selected from the group consisting of:

- 20 (a) the nucleotide sequence of SEQ ID NO:7;
- (b) the nucleotide sequence of SEQ ID NO:8;
- 25 (c) a nucleic acid molecule which is at least 80% identical to the nucleic acid of (a) or (b) and which encodes a peptide that is capable of binding to an intracellular receptor region of an α -subunit of a voltage-gated ion channel; and
- (d) a nucleic acid molecule which is degenerate as a result of the genetic code to a nucleic acid defined in (a) or (b) and which encodes a peptide that is
- 30 capable of binding to an intracellular receptor region of an α -subunit of a voltage-gated ion channel.

41. An expression vector comprising the polynucleotide of claim 39.

42. An expression vector comprising the polynucleotide of claim 40.
43. A host cell transfected or transformed with the expression vector of
5 claim 41.
44. A host cell transfected or transformed with the expression vector of
claim 42.

2025-10-10 14:50:00